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**BIOENERGY TECHNOLOGIES OFFICE** 

DOE Bioenergy Technologies Office (BETO) 2023 Project Peer Review

# **Process and Integration Scale Up Task**

April 3, 2023
Technology Area Session: Conversion

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## **Project Goal and BETO relevance**

### **Goal of Process and Integration Scale Up Task**

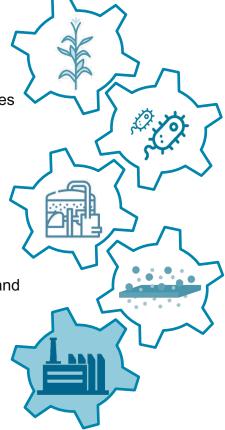
- Conduct bioreactor cultivations for ABF Target-Host pairs across scales, integrate with Test activities
- Expand and maintain bioreactor equipment across the ABF
- Produce and deliver hydrolysate to partners and industry
- Act as a liaison between ABF and other BETO consortia (i.e. Separations Consortium)
- Provide integrated data to technoeconomic analyses and life cycle assessment teams

#### **Relevance to BETO's Goals**

 Benchmark DBTL cycle performance and improvement across scales with real-world substrates and process configurations

### **Specific Research Question**

• How robust are the biocatalysts in industrially relevant setups and at scale? How feasible are the bioprocesses at scale? How can we improve the performance of the biocatalysts while developing bioprocesses?





## **Approach**

### **Bioprocess development for DBTL activities**

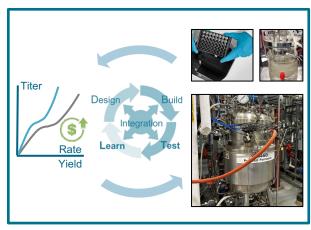
- Develop media, feeding strategies, and process control to improve product titers, rates, and yields
- Conduct Test experiments at different scales (from 1 mL to 1,500 L scale)
- Evaluate strains in process relevant conditions
- Key outcomes are to identify strain bottlenecks, improve production in industrially relevant conditions, and develop scalable processes
- Produce and deliver industrially relevant lignocellulosic hydrolysate to ABF partners

**Challenge:** achieve product metrics (titers, rates, or yields) that are industrially relevant

**Risks/Mitigation:** Oxygen transfer demands and contamination issues at large scale/ support scale up activities with computational fluid dynamic models and sensitivity analysis to understand the effect of contamination on the overall process

**Risks/ Mitigation:** Product metrics are not achieved due to the toxicity of the compound or salts added in the bioreactors/ Strain development based on these challenges & Collaborate with the Separations Consortium to develop *in situ* product recovery systems









## **Approach**

### Interaction with other ABF tasks, other consortia, and industry

- This task provides realistic, relevant data to Integrated Analysis team
- Engagement with other BETO projects to amplify ABF impact through integration (e.g. Separations Consortium to improve product recovery and Feedstock Conversion Interface Consortium to understand the effect of feedstock variability on Hosts
- Engagement with industry via Direct Funding Opportunities (DFOs)

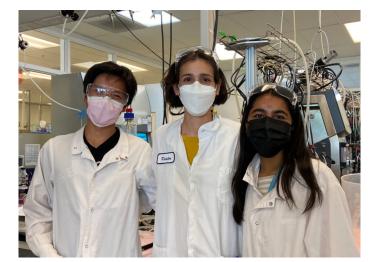
### Management, communication, & DEI

- Meetings: bi-weekly with PI-task leads
- Ad hoc meetings with other BETO projects
- Active recruiting of students (GEM program), team diversity
- Mentoring of High-school interns through Pilot City Program at ABPDU@LBNL
- Focused on creating physically and psychologically safe research environments

### **Exemplary milestone with technical metrics:**

FY21-Q4. Demonstrate TRY of 40 g/L, 0.5 g/L/h, 60% theoretical yield from DMR-EH hydrolysate or mock hydrolysate (achieved with the target-host pair muconate-*P.putida*)

## Go/No-Go Decision: N/A



Pilot City Internship Program for High School students: Tyler Pham, ABF Project Scientist Kristen Hunter-Cavera, and intern Sanjana Rathore worked on *R. toruloides* in Summer for 2022





## Approach: Equipment and capabilities

### **Integrated Biorefinery Research Facility** (IBRF)

#### **Bioreactor capabilities**

#### Bench scale

- BiolectorPro (2 mL)
- Robolector (2 mL)
- 0.5 L x 36
- 1 L x 6
- 3 L x 6
- 8 L x 2 (designed for high solids content)
- 10 L x 4

#### Pilot plant

- 30 L x 1
- 150 L x 2
- 1.500 L x 2
- 9,000 L x 4





### Other capabilities

#### Off-gas mass spectrometer

**Off-line real-time metabolite analyzers** (YSI analyzers)

In situ product recovery (ISPR) (Liquid-liquid extraction system coupled to 3 L bioreactor)

Rotary ceramic disk (cell/broth separation at the micro- and nano- scale in pilot plant)

### **Advanced Biofuels and Bioproducts Process Development Unit (ABPDU)**

### **Bioreactor capabilities**

#### Bench scale

- Biolector 48 wells up to 2.5 mL
- Sartorius Ambr 12 reactors X 0.25 L
- Sartorius BioStat 4 reactors X 2 L
- Eppendorf 1 reactor X 10 L
- ABEC 1 reactor X 50 L and 2 reactors X 300L

#### Gas Fermentation/ Pressurized Fermentations

- 500 mL HEL reactors with manifold for multi-gas supply
- 20L HEL reactor (commissioning in progress)

#### Other capabilities

#### Off-gas mass spectrometer

Off-line real-time metabolite analysis with Gallery™

Pilot-scale decanting and disc stack centrifuge

Tangential Flow Filtration and Rotatory Evaporation

Deconstruction and advanced analytical capabilities

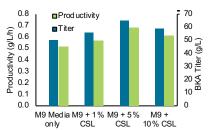




PISU supports partners within the ABF, ABF-DFOs, and other consortia

#### Bioprocess development for various products by engineered P. putida from sugars

(1) β-Ketoadipate from glucose and xylose 65.2 g/L, 0.68 g/L/h and 0.40 C-mol/C-mol



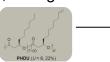
#### Poly-(R)-(3-hydroxydecanonate-co-3hydroxyundecenoate) (PHDU) with different levels of unsaturation

(2) PHDU-6: 5.6 g/L, 0.05 g/L/h (Cywar, Chen et al, in review)

(3) PHDU-22: 4 g/L, 0.04 g/L/h











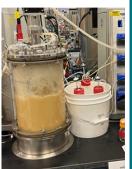
Milestones FY21-Q3: Provide three additional compounds of interest to BETO's Performance-Advantaged BioProducts project at sufficient quantities for polymer testing (10 g at 1-2 g/L). Result: accomplished

#### Production of chemical precursors for downstream purification and testing

- Deliver to DFO partner
- β-ketoadipate production from glucose and xylose (10 L scale)



**Milestones** (FY22): Produce  $> 100 g of \beta$ ketoadipate and deliver to Technology Holding Result: Delivered.



#### **Developing in situ product** recovery (ISPR) systems

- Collaboration with Separations Consortium (SepCon)
- Production of muconate and βketoadipate (10 L scale) using various media compositions



**Milestones** (SepCon) (FY23): Evaluate ISPR systems based on adsorption to recover chemical precursors Result: We delivered product on time





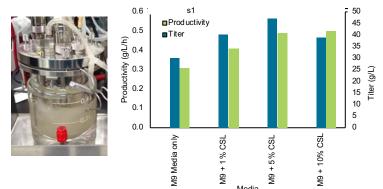


#### Developing industrially relevant processes for muconate production from sugar hydrolysates



#### Bioprocess development in 0.5 L bioreactors

- Muconate from glucose and xylose by engineered P. putida
- Muconate titer (47.2 g/L), yield (0.52 C-mol/C-mol glucose and xylose) (>95% theoretical yield), rate (0.49 g/L/h)



Milestones (FY21-Q4): Demonstrate TRY of 40 g/L, 0.5 g/L/h, 60% theoretical yield from DMR-EH hydrolysate or mock hydrolysate.

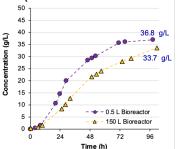
Result: showed 47 g/L at 0.5 g/L/h and >90% theoretical yield.



#### **Scale up (150 L)**

Our process is scalable to 150 L (first successful demonstration at scale)





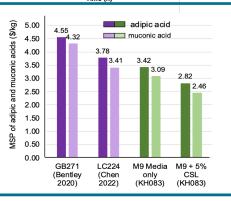


#### **TEA and LCA**

- Muconate MSP 2.46 \$/kg
- Adipate MSP 2.82 \$/kg

Milestones (FY22-Q4): Calculate the minimum selling price of chemicals to benchmark realistic chemical markets to target from a metabolism perspective.

Result: PISU delivered data to TEA team to hit this milestone



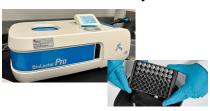


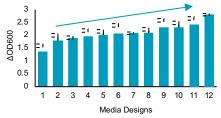


### Media development for P. putida

### Machine learning applied to media development

- Opentron (automatic media preparation) + Biolector
- ART tool (developed in LBNL) to recommend media design
- Numerous DBTL cycles





## Evaluation of *P. putida* strains in 0.5 L bioreactors

- Glucose and xylose to muconate (Chen et al, Nat Comm, 2022)
- Glucose and xylose to β-ketoadipate (3 strains)
- Glucose, xylose, arabinose to muconate (6 strains)

#### LANL

**NREI** 

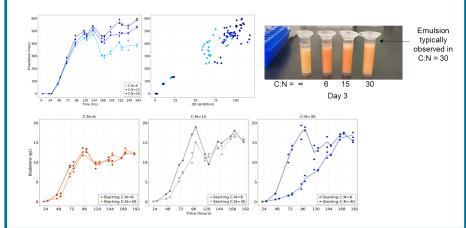
Glucose to muconate (~15 strains)

#### **ANL**

Glucose to muconate (3 strains)

### Media development for *Rhodotorula toruloides*

- Developed new minimal media recipe for R. toruloides to effectively test C:N ratio
- Conducted bioreactor (Ambr system) campaigns with both bisabolene and prespatane producing strains
- Variability observed from starting ratio as well as feed ratio
- Other cell aspects affected from C:N ratios









### Illustrate better utilization of real-time data for in-line process control

#### **Bioreactor real-time Sensing Capabilities**

- ABF co-lead participated in DOE's I-Corp program where we learned about sensing issues in bioreactors.
- We applied these learnings by developing a "soft-sensor" to control feed rate for a fermenter.
- We chose to focus on oxygen utilization rate (OUR) as it represents the amount of oxygen being consumed and thereby reflects on cell concentration and metabolic activity.
- OUR is more informative metric than dissolved oxygen alone and has the advantage of being utilized across different reactor vessels and scales.
- ABPDU Engineers utilized this metric to manually adjust feed rate or stirrer agitation in real-time for several industry projects and aim to apply this approach for an ABF 300L campaign in the futures.

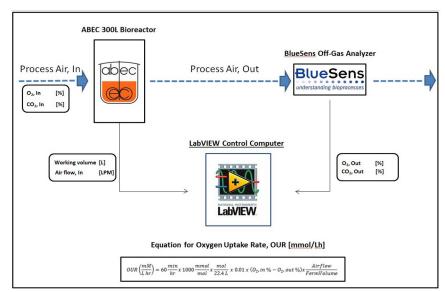


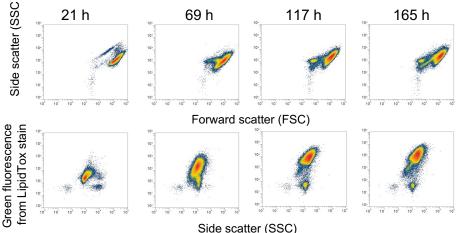
Diagram of OUR process control. Air flow coming into 300L reactor was monitored by ABEC software as was total reactor volume. BlueSens Analyzer monitored gas percentages in off gas. These values were utilized by LabView control software to provide real time OUR, according to equation listed above.







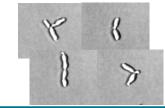
Investigation with flow cytometry to better understand cell physiological state and population heterogeneity in bioreactor cultivations



 Changes in Side scatter (SSC) and Forward scatter (FSC) signatures on R. toruloides

Accumulation of lipid droplets

Healthy budding cells on Day 1



8 0

Deteriorating cells on Day 5

Data to feed into ABPDU AOP Task #2

– Image analysis to study microbial
heterogeneity in real-time and assess
how process conditions are affecting
cell health and thereby TRY metrics





### **PISU Milestones from FY21/FY22**



- FY21-Q4. Demonstrate TRY of 40 g/L, 0.5 g/L/h, 60% theoretical yield from DMR-EH hydrolysate or mock hydrolysate (see slide 7)
- **FY21Q3\_PISU\_R2.** Provide three additional compounds of interest to BETO's Performance-Advantaged BioProducts mini-consortium at sufficient quantities for polymer testing (10 g at 1-2 g/L) (see slide 6)
- FY22Q2\_PISU\_R1. Better utilization of real-time data for in-line process control demonstrated. At least a single non-traditional parameter integrated to control fermenter feed rate. Target metic achieved: Oxygen uptake rates were calculated in real-time from off-gas data and applied to control feed-rate and agitation dynamically. (see slide 9)
- FY22Q3\_PISU\_R1 Provide three additional compounds of interest to BETO's Performance-Advantaged BioProducts projects at sufficient quantities for property testing (see slide 6)





## **Impact**

### **Scientific**

- Demonstrates Foundry strain performance on industrially relevant substrates
- Self-consistent testing mechanisms
- Public information on scale up, scale down cultivations in bioreactors as well as non-model strain performance on lignocellulosic substrates
- Technology transfer activities, patent applications
- Additional learnings on hosts/processes due to downstream processing with other BETO consortia





### **Dissemination**

- Presentations in conferences (Symposium on Biomaterials, Fuels and Chemicals)
- Publications (C Ling, et al. (2022) Nature Communications; Rorrer et al. (2022)
   Cell Reports Physical Science)
- Engagement with industry and other BETO consortia





## **Impact**



- We have identified key cost drivers (PISU & TEA tasks)
- Improved Minimum Selling Price for different products (e.g. muconic acid MSP (direct replacement for adipic acid) compared to adipic acid MSP from petrochemical industry)
- Industry collaborations through DFOs



**β-Ketoadipate** 65.2 g/L, 0.68 g/L/h and 0.40 C-mol/C-mol





Demonstrated loss in TRY depending on bioreactor stressor





Just Completed Contracting



**Just Initiated** 



In Contracting Phase



33.7 g/L in P. putida at lab-scale; Biosensor development in progress, Enduro Bacillus strains outperformed wildtype at 300L





## **Summary**

#### **Overview**

Provide data (bioreactor performance across scales) as part of DBTL efforts

### **Approach**

- Driving progress with holistic approaches and state-of-the-art process development
- Interface with ABF tasks, BETO projects, and industry

### **Progress and outcomes**

- Improved strain performance through bioreactor process development (e.g. 47 g/L muconate, 65 g/L β-ketoadipate)
- Successfully scaled up a Target-Host pair (150 L, Muconate-*P. putida,* 34 g/L muconate)
- Generated a diversity of products from a variety of Target-Host pairs (e.g. PHDU-P. putida, 5.6 g/L & 3HP- R. toruloides, 45.4 g/L)
- Produced lignocellulosic hydrolysates

#### **Impact**

- Advancing the feasibility of scaling non-model hosts
- Enabling industry collaborations via DFO projects











# **Additional Slides**

### Publications, Patents, Presentations, Awards, and Commercialization

#### 70 publications, 189 presentations to date

- 54 publications and 115 presentations since FY2019
- The following slides list these publications and presentations

#### 17 patents, 10 records of invention, 9 software disclosures, & 2 licenses

• The following slides list these intellectual property assets





- Garima Goyal, Zak Costello, Jorge Alonso Guitierrez, Aram Kang, Taek Soon Lee, Hector Garcia Martin, and Nathan J Hillson. (2018) "Parallel Integration and Chromosomal Expansion of Metabolic Pathways" ACS Synthetic Biology DOI: 10.1021/acssynbio.8b00243
- Jha RK, Narayanan N, Pandey N, Bingen JM, Kern TL, Johnson CW, Strauss CEM, Beckham GT, Hennelly SP, Dale T. Sensor-enabled alleviation of product inhibition in chorismate pyruvate-lyase. ACS Synthetic Biology (2019), 10.1021/acssynbio.8b00465.
- Oyetunde, T., Liu, D., Martin, H. G., & Tang, Y. J. "Machine learning framework for assessment of microbial factory performance." PloS one 14.1: e0210558 (2019).
- Ando, D., and Garcia Martin, H. "Genome-Scale 13 C Fluxomics Modeling for Metabolic Engineering of Saccharomyces cerevisiae" Microbial Metabolomics. Humana Press, New York, NY: 317-345 (2019).
- Costello, Zak, and Hector Garcia Martin. "How to Hallucinate Functional Proteins." arXiv preprint arXiv:1903.00458 (2019).
- Chen Y, Vu J, Thompson MG, Sharpless WA, Chan LJG, et al. (2019) A rapid methods development workflow for high-throughput quantitative proteomic applications. PLOS ONE 14(2): e0211582. https://doi.org/10.1371/journal.pone.0211582





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- Jesus F. Barajas, Ryan P. McAndrew, Mitchell G. Thompson, Tyler W. H. Backman, Bo Pang, Tristan de Rond, Jose H. Pereira, Veronica T. Benites, Héctor García Martín, Edward E. K. Baidoo, Nathan J. Hillson, Paul D. Adams, and Jay D. Keasling. (2019) "Structural insights into dehydratase substrate selection for the borrelidin and fluvirucin polyketide synthases" J Ind Microbiol Biotechnol https://doi.org/10.1007/s10295-019-02189-z
- Jesus F. Barajas, Maren Wehrs, Milton To, Lauchlin Cruickshanks, Rochelle Urban, Adrienne McKee, John Gladden, Ed-Been-Goh, Margaret E. Brown, Diane Pierotti, James M. Carothers, Aindrila Mukhopadhyay, Jay D. Keasling, Jeffrey L. Fortman, Steven W. Singer, Constance B. Bailey. (2019) "Isolation and characterization of bacterial cellulase producers for biomass deconstruction: A microbiology laboratory course" J Microbiology and Biology Education. Just accepted. https://doi.org/10.1128/jmbe.v20i2.1723
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- Carbonell, P., Radivojevic, T. and Garcia Martin, H., 2019. Opportunities at the Intersection of Synthetic Biology, Machine Learning, and Automation. ACS Synthetic Biology 1474-1477 (2019)
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- Tijana Radivojevic, Elena Akhmatskaya, "Modified Hamiltonian Monte Carlo for Bayesian inference", Statistics and Computing, https://doi.org/10.1007/s11222-019-09885-x
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- Christopher B. Eiben, Tristan de Rond, Clayton Bloszies, Jennifer Gin, Jennifer Chiniquy, Edward E. K. Baidoo, Christopher J. Petzold, Nathan J. Hillson, Oliver Fiehn, Jay D. Keasling. (2019) "Mevalonate Pathway Promiscuity Enables Noncanonical Terpene Production", ACS Synth. Biol. https://doi.org/10.1021/acssynbio.9b00230
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   "Systems and synthetic biology tools for advanced bioproduction hosts", Curr. Op. Biotechnol. https://doi.org/10.1016/j.copbio.2019.12.007





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  Thompson, Jesus F. Barajas, Jeffrey Chen, Rohith N. Krishna, Leanne Jade G. Chan, Danika
  Nimlos, Catalina Alonso-Martinez, Edward E. K. Baidoo, Yan Chen, Jennifer W. Gin, Leonard
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- Isabel Pardo‡, Ramesh Jha‡, Molly Gaddis, Ryan Bermel, Felicia Bratti, Molly Gaddis, Emily McIntyre, William E. Michener, Ellen L. Neidle, Taraka Dale, Gregg T. Beckham\*, Christopher W. Johnson\*, Gene amplification, laboratory evolution, and biosensor screening reveal MucK as a terephthalic acid transporter in Acinetobacter baylyi ADP1, Metabolic Eng. (2020), 62, 260-274.
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- Chen Ling, George L. Peabody, Davinia Salvachúa, Young-Mo Kim, Colin M. Kneucker, Michela A. Monninger, Nathalie Munoz, Brenton C. Poirier, Kelsey J. Ramirez, Peter C. St. John, Sean P. Woodworth, Jon K. Magnuson, Kristin E. Burnum-Johnson, Gregg T. Beckham\*, Adam M. Guss,\* Christopher W. Johnson,\* Muconic acid production from glucose and xylose in Pseudomonas putida KT2440 via evolution and metabolic engineering, in review at Nature Comm.





- Nicholas A. Rorrer,‡ Sandra F. Notonier,‡ Brandon C. Knott,‡ Brenna A. Black,‡, Avantika Singh,‡ Scott R. Nicholson,‡ Christopher P. Kinchin, Graham P. Schmidt, Alberta C. Carpenter, Kelsey J. Ramirez, Christopher W. Johnson, Davinia Salvachúa, Michael F. Crowley, Gregg T. Beckham\*, Production of B-ketoadipic acid from glucose in Pseudomonas putida KT2440 for use in performance-advantaged nylons, in review at Cell Reports Phys. Sci.
- Precise genomic riboregulator control of metabolic flux in microbial systems. Naresh Pandey, Steffi A. Davison, Malathy Krishnamurthy, Daniel Trettel, Chien-Chi Lo, Shawn Starkenburg, Katherine L. Wozniak, Theresa Kern, Sean D. Reardon, Clifford J. Unkefer, Scott P. Hennelly and Taraka Dale. Submitted to ACS Synthetic Biology December 2021.
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- Hyun Gyu Lim, Kevin Rychel, Anand V. Sastry, Gayle J. Bentley, Joshua Mueller, Heidi S. Schindel, Peter E. Larsen, Philip D. Laible, Adam M. Guss, Wei Niu, Christopher W. Johnson, Gregg T. Beckham, Adam M. Feist, Bernhard Palsson, Machine-learning from Pseudomonas putida KT2440 transcriptomes reveals its transcriptional regulatory network, Metabolic Eng. (2022) 72, 297-310.
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- Hector Garcia Martin\*, Tijana Radivojevic, Jeremy Zucker, Kristofer Bouchard, Jess Sustarich, Sean Peisert, Dan Arnold, Nathan Hillson, Gyorgy Babnigg, Jose Manuel Marti, Christopher J. Mungall, Gregg T. Beckham, Lucas Waldburger, James Carothers, ShivShankar Sundaram, Deb Agarwal, Blake A. Simmons, Tyler Backman, Deepanwita Banerjee, Deepti Tanjore, Lavanya Ramakrishnan, Anup Singh, Perspectives for self-driving labs in synthetic biology, submitted to Curr. Opin. Biotech
- N. Pandey, S.A. Davison, M. Krishnamurthy, D.S. Trettel, C. Lo, S. Starkenburg, K.L. Wozniak, T.L. Kern, S.D. Reardon, C.J. Unkefer, S.P. Hennelly and T. Dale. 2022. Precise genomic riboregulator control of metabolic flux in microbial systems. ACS Syn. Biol https://doi.org/10.1021/acssynbio.1c00638





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- Kyle R. Pomraning; Shuang Deng; Joonhoon Kim; Kristen B. Campbell; Ana L. Robles; Beth Hofstad; Nathalie Munoz; Yuqian Gao; Teresa Lemmon; Marie S. Swita; Jeremy D. Zucker; Young-Mo Kim; Kristin E. Burnum-Johnson; Jon K. Magnuson; Ziyu Dai. 2022 "Metabolic engineering to improve production of 3-hydroxypropionic acid from corn-stover hydrolysate in Aspergillus species." Metabolic Engineering
- Kevin J. McNaught, Eugene Kuatsjah, Michael Zahn, Érica T. Prates, Huiling Shao, Gayle J. Bentley, Andrew R. Pickford, Josephine N. Gruber, Kelley V. Hestmark, Daniel A. Jacobson, Brenton C. Poirier, Chen Ling, Myrsini San Marchi, William E. Michener, Carrie D. Nicora, Jacob N. Sanders, Caralyn J. Szostkiewicz, Dušan Veličković, Mowei Zhou, Nathalie Munoz, Young-Mo Kim, Jon K. Magnuson, Kristin E. Burnum-Johnson, K.N. Houk, John E. McGeehan, Christopher W. Johnson, Gregg T. Beckham, "Initiation of fatty acid biosynthesis in Pseudomonas putida KT2440", Metabolic Engineering, Volume 76, 2023, Pages 193-203, https://doi.org/10.1016/j.ymben.2023.02.006





- Gregg Beckham, Hybrid biological and catalytic processes to manufacture and recycle plastics,
   Princeton University, November 28th, 2018
- Nathan J. Hillson. "DOE Agile BioFoundry Overview". Invited Talk, SynBioBeta 2018 visit to ESE, Emeryville, CA, October 1, 2018
- Nathan J. Hillson. "Recent developments at the U.S Department of Energy Agile BioFoundry".
   Invited Talk, 2nd Darmstadt RoboWorkshop, Darmstadt, Germany, November 7, 2018
- Nathan J. Hillson. "DIVA (DNA Design, Implementation, Validation Automation) Platform".
   Invited Talk, 2nd Darmstadt RoboWorkshop, Darmstadt, Germany, November 8, 2018
- Garcia Martin, H. "Towards a predictive synthetic biology enabled by machine learning and automation". Ginkgo Bioworks, Boston, MA, November 12, 2018; AIChE annual meeting, Pittsburgh, PA, October 31 2018; Thermo Fisher, San Jose, CA, October 19, 2018; DTRA Tech Watch, Ft. Belvoir, VA, October 10, 2018.
- Garcia Martin, H. "A New Approach to Flux Analysis". ABF Annual Meeting, Berkeley CA, September 7, 2018.
- Nathan J. Hillson. "BioDesign Department Overview". Invited Talk, BSE Annual Meeting, Berkeley, CA, January 24, 2019





- Nathan J. Hillson. "Agile BioFoundry Overview". Invited Talk, BETO Peer Review, Denver, CO, March 7, 2019
- Nathan J. Hillson. "Agile BioFoundry DBTL Infrastructure". Invited Talk, BETO Peer Review, Denver, CO, March 7, 2019
- Nathan J. Hillson. "DNA Synthesis Science at the U.S. DOE Joint Genome Institute: Biosecurity Sequence Screening and Broader Aspects Review". Invited Talk, EBRC Spring Retreat 2019, Boston, MA, March 23, 2019
- Nathan J. Hillson. "ABF: plans for the next 3-year cycle". Invited Talk, BETO Quarterly FY19Q2, Washington DC, March 27, 2019
- Jennifer Chiniquy. "Emery Station East DIVA DNA Sequencing". Invited Talk, BSE Annual Meeting, Berkeley, CA, January 24, 2019
- Garcia Martin, H. "Towards a predictive synthetic biology enabled by machine learning and automation". EmeryStation Campus, Emeryville, CA, February 7th, 2019.
- Garcia Martin, H. "Metabolic modeling, drug synthesis, and their interaction". LBNL Biosciences Experts Advisory Committee, Berkeley, CA, February 8th, 2019.
- Garcia Martin, H. "Towards a predictive synthetic biology enabled by machine learning and automation". Google X, Mountain View, CA, February 14th, 2019.





- Garima Goyal, Z. Costello, J.A. Gutierrez, A. Kang, T.S. Lee, H.G. Martin, and N.J. Hillson.
   "PIACE: Parallel Integration and Chromosomal Expansion of metabolic Pathways". Invited Talk, ACS Conference, Orlando, Florida, April 4, 2019
- Nathan J. Hillson. "DNA synthesis use and biosecurity screening at U.S. DOE projects including the Joint Genome Institute, Joint BioEnergy Institute, and Agile BioFoundry". Invited Talk, Gene Synthesis Governance Meeting, Johns Hopkins Center for Health Security, St. Regis Hotel, Washington, DC, April 9, 2019
- Nathan J. Hillson. "DOE Agile BioFoundry: Overview and Recent Highlights". Invited Talk, SBFC 2019 Session ST-2: Global Research Consortia, Seattle, WA, May 1, 2019
- Nathan J. Hillson. "2019 BETO Merit Review Process". Invited Talk, ABF IAB FY19Q3, Zoom Videoconference, May 17, 2019
- Nathan J. Hillson. "Machine Learning-assisted MiSeq library loading". Invited Talk, ABF IAB FY19Q3, Zoom Videoconference, May 17, 2019
- Nathan J. Hillson. "Global Biofoundries Alliance: 2019 Annual Meeting Report Out". Invited Talk, ABF IAB FY19Q3, Zoom Videoconference, May 17, 2019
- Nathan J. Hillson. "2019 BETO Peer Review Report Out". Invited Talk, ABF IAB FY19Q3, Zoom Videoconference, May 17, 2019





- Nathan J. Hillson. "ICE/DIVA, EDD, and ART". Invited Talk, Software for Synthetic Biology Workflows Workshop, SEED 2019, New York, NY, June 27, 2019
- Nathan J. Hillson. "DOE Agile BioFoundry: Overview and Recent Highlights". Invited Talk, JBEI Annual Meeting 2019, Monterrey, CA, May 30, 2019
- Henrique C. De Paoli. "Overview of Synthetic Biology principles". Invited Talk, Xu's Research Group, UC Dept. of Materials Science and Engineering, Berkeley, CA, May 13, 2019.
- Nathan J. Hillson "ABF: CRADA updates and FY20-22". Invited Talk, BETO Conversion Call, July 1, 2019
- Wei Xiong and Nathan Hillson, "Synthetic C1 Condensation Cycle for Formate-Mediated ElectroSynthesis". Invited Talk, BETO Conversion Call, July 8, 2019
- Ernst Oberortner, Nathan J. Hillson, and Jan-Fang Cheng. "The Operon Refactoring and Construction Assistant (ORCA): Streamlined gene cluster refactoring". Invited Talk, 11th International Workshop on Bio-Design Automation, University of Cambridge, UK July 9, 2019
- Nathan J. Hillson "ABF Overview, FY19 Update, and FY20-22 Plans". Invited Talk, ABF Annual Meeting, Richland, WA, July 30, 2019
- Nathan J. Hillson "FY22Q4\_DBTL\_AS1: 5x efficiency improvements". Invited Talk, ABF Annual Meeting, Richland, WA, July 31, 2019





- Nathan J. Hillson "DOE Agile BioFoundry: Overview and Recent Highlights". Invited Talk, VIP Visit - Wendy Pulling | Director of ESG Integration University of California Office of the Chief Investment Officer, Emeryville, CA, Aug 6, 2019
- Nathan J. Hillson "DOE Agile BioFoundry: Overview and Recent Highlights". Invited Talk, Tiangong Forum Distinguished Lecture, Tianjin Institutes of Industrial Biotechnology (TIB), Chinese Academy of Sciences (CAS), Tianjin, China, August 16, 2019
- Nathan J. Hillson "DOE Agile BioFoundry: DBTL Infrastructure". Invited Talk, SynBioYSF 2019, Tianjin Institutes of Industrial Biotechnology (TIB), Chinese Academy of Sciences (CAS), Tianjin, China, August 17, 2019
- Nathan J. Hillson "U.S. DOE Agile BioFoundry: Overview and Recent Highlights", Invited Talk, Genscript Double Helix Symposium 2019, San Francisco, CA September 30, 2019
- Jennifer Chiniquy "DIVA DNA Sequencing". Invited Talk, 2019 ABF All Hands Meeting, Pacific Northwest National Laboratory, Richland, WA, July 30, 2019.
- Tijana Radivojevic, "Automatic Recommendation Tool", Invited Talk, ABF Annual Meeting 2019, Richland, WA, July 30, 2019
- Nurgul Kaplan."Automated DNA Construction: "from j5 protocol design to Laboratory Robotics" Invited Talk, ABF Annual Meeting 2019, Pasco, WA, July 30, 2019





- Nathan J. Hillson "U.S. DOE Agile BioFoundry: Organization and Capabilities", Invited Talk, ABF Industry Day 2019, Emeryville, CA October 4, 2019
- Garcia Martin, H. "Machine Learning, Synthetic Biology and Automation: Engineering Life for the Benefit of Society". NERSC data seminar, Berkeley CA, November 1st, 2019.
- Garcia Martin, H. "ART: a machine learning Automated Recommendation Tool for guiding synthetic biology". Al4Synbio Symposium, Arlington VA, November 8th, 2019.
- Garcia Martin, H. "Opportunities in the intersection of:Artificial Intelligence & Synthetic Biology & Automation". Army Science Planning and Strategy Meeting, Burlington MA, November 13th, 2019.
- Nathan J. Hillson "ABF: CRADA updates and FY20-22". Invited Talk, BETO Conversion Call, July 1, 2019
- Wei Xiong and Nathan Hillson, "Synthetic C1 Condensation Cycle for Formate-Mediated ElectroSynthesis". Invited Talk, BETO Conversion Call, July 8, 2019
- Ernst Oberortner, Nathan J. Hillson, and Jan-Fang Cheng. "The Operon Refactoring and Construction Assistant (ORCA): Streamlined gene cluster refactoring". Invited Talk, 11th International Workshop on Bio-Design Automation, University of Cambridge, UK July 9, 2019





- Nathan J. Hillson "ABF Overview, FY19 Update, and FY20-22 Plans". Invited Talk, ABF Annual Meeting, Richland, WA, July 30, 2019
- Nathan J. Hillson "FY22Q4\_DBTL\_AS1: 5x efficiency improvements". Invited Talk, ABF Annual Meeting, Richland, WA, July 31, 2019
- Nathan J. Hillson "DOE Agile BioFoundry: Overview and Recent Highlights". Invited Talk, VIP Visit - Wendy Pulling | Director of ESG Integration University of California Office of the Chief Investment Officer, Emeryville, CA, Aug 6, 2019
- Nathan J. Hillson "DOE Agile BioFoundry: Overview and Recent Highlights". Invited Talk, Tiangong Forum Distinguished Lecture, Tianjin Institutes of Industrial Biotechnology (TIB), Chinese Academy of Sciences (CAS), Tianjin, China, August 16, 2019
- Nathan J. Hillson "DOE Agile BioFoundry: DBTL Infrastructure". Invited Talk, SynBioYSF 2019, Tianjin Institutes of Industrial Biotechnology (TIB), Chinese Academy of Sciences (CAS), Tianjin, China, August 17, 2019
- Nathan J. Hillson "U.S. DOE Agile BioFoundry: Overview and Recent Highlights", Invited Talk, Genscript Double Helix Symposium 2019, San Francisco, CA September 30, 2019
- Jennifer Chiniquy "DIVA DNA Sequencing". Invited Talk, 2019 ABF All Hands Meeting, Pacific Northwest National Laboratory, Richland, WA, July 30, 2019.





- Tijana Radivojevic, "Automatic Recommendation Tool", Invited Talk, ABF Annual Meeting 2019, Richland, WA, July 30, 2019
- Nurgul Kaplan."Automated DNA Construction: "from j5 protocol design to Laboratory Robotics"
   Invited Talk, ABF Annual Meeting 2019, Pasco, WA, July 30, 2019
- ART: A machine learning Automatic Recommendation Tool for guiding synthetic biology", Invited Talk, Computational Bio-Science Meeting, Berkeley, CA, April 23, 2020
- Garcia Martin, H. "Opportunities in the intersection of machine learning, synthetic biology, and automation". ABLC 2020, Virtual meeting, July 10th, 2020.
- Garcia Martin, H. "Leveraging machine learning and automation to make synthetic biology predictable". SPIE Optics + Photonics 2020, Virtual meeting, August 24th, 2020.
- Nathan J. Hillson, "FY20 ABF CRADA Call: Process, Applications, and Selections", Conversion R&D Standing Lab Update Call, via WebEx, July 27, 2020
- Nathan J. Hillson, "Perspectives from the U.S. DOE Agile BioFoundry", OECD BNCT Virtual Workshop, Session 1: Biofoundries and COVID-19, via Zoom, July 29, 2020
- Nathan J. Hillson, "DIVA, EDD, and ART: Software spanning the Design/Build/Test/Learn cycle", invited talk, COMBINE 2020, via Zoom, October 5, 2020





- Nathan J. Hillson, "DIVA, EDD, and ART: Software spanning the Design/Build/Test/Learn cycle", invited talk, SPARC Workshop (IIT Kharagpur) 2020, via Zoom, October 19, 2020
- Invited talk: Guss AM. "Domestication of non-model microbes for the production of renewable fuels and chemicals" (2021) The Metabolic Engineering Virtual Seminar Series at the University of Texas-Austin. Virtual.
- Garcia Martin, H. "Leveraging machine learning and automation to make bioengineering predictive". The Metabolic Engineering Virtual Seminar, UT Austin, February 12th, 2021.
- Garcia Martin, H. "Machine Learning for Bioengineering". JBEI Board of Directors, March 12th, 2021.
- Nathan J. Hillson, "ABF Overview", invited talk, BETO Peer Review 2021, via Zoom, March 9, 2021
- Nathan J. Hillson, "ABF DBTL Infrastructure", invited talk, BETO Peer Review 2021, via Zoom, March 9, 2021
- Nathan J. Hillson, "ABF Overview", invited talk, BSA ExComm, via Zoom, March 12, 2021
- Guss AM. "Synthetic biology and metabolic engineering of non-model microbes for the production of renewable fuels and chemicals" Seminar at Purdue University Northwest. April 16, 2021.





- Radivojevic, T., "Automated Recommendation Tool (ART): Design of Experiments via Probabilistic Predictive Modeling", Autonomous Discovery in Science and Engineering Workshop, April 21, 2021(Invited Tutorial)
- Radivojevic, T., "Guiding synthetic biology via machine learning", Symposium on Biomaterials,
   Fuels and Chemicals (SBF): Speeding up synthetic biology, April 27, 2021
- Radivojevic, T., "Guiding synthetic biology via Automated Recommendation Tool (ART)", Synthetic Biology: Engineering, Evolution & Design (SEED) 2021, Computation, Artificial Intelligence, and Machine Learning for Biological Design Session, June 17, 2021 (Invited Speaker)
- Guss AM. "Domestication of non-model microbes for the production of renewable fuels and chemicals" Student-invited seminar at North Carolina State University. September 27, 2021.
- Radivojevic, T. "Guiding synthetic biology via machine learning and multi-omics technologies", IWBDA 2021, 13th International Workshop on Bio-Design Automation, September 20, 2021 (virtual)
- Hillson, Nathan J. "Session Introduction", Metabolic Engineering 14, Block 8: Session Biofoundries, (Virtual via Remo), July 15, 2021





- Hillson, Nathan J. "Updates on DOE assets including Agile BioFoundry, Joint BioEnergy Institute, and Joint Genome Institute", Digital Biology Engineering Meeting (Air Force) via zoom, September 23, 2021
- Garcia Martin, H. "Machine Learning Tools Can Make Synthetic Biology Predictable". SIMB, August 9th, 2021.
- Garcia Martin, H. "Leveraging machine learning and automation to make bioengineering predictable". Indo-US Workshop on Application of Data Science in Biological Systems, September 7th, 2021.
- Garcia Martin, H. "Leveraging machine learning and automation to make bioengineering predictable". Air Force Research Laboratory MIrACLE Forum, September 10th, 2021.
- Garcia Martin, H. "Challenges and opportunities in high-throughput data synthesis". NSF
  Challenges and Opportunities in Synthesizing Massively Parallel Assays and High-Throughput
  Datasets Workshop, September 17th, 2021.
- Garcia Martin, H. "Guiding metabolic engineering via kinetic deep learning and multi-omics".
   Quantitative Modelling of Cell Metabolism Conference, September 21th, 2021.
- Davison, S. Reprogramming Microbes for Biomanufacturing, Science in 3 at Los Alamos National Laboratory, September 2021





- Wozniak, K. Engineering a Sustainable Future: Fine-tuning Gene Expression for Biomanufacturing, Science in 3 at Los Alamos National Laboratory, September 2021
- Davison, S. Fine-tuning gene expression in bioproduction pathways in diverse bacterial hosts, SIMB Annual Meeting, August.
- Wozniak, K. Targeting global regulatory responses using precise and programmable cisriboregulators SIMB Annual Meeting, August
- Adam Guss. "Genetic tools and microbial engineering for biological production of sustainable fuels and chemicals" Presented to Weekly Seminar for DOE CCI/SULI Students. October 27, 2021
- Adam Guss. "Domestication of diverse non-model microbes for plastics upcycling and sustainable fuel and chemical production" Biological Sciences Departmental Seminar, Michigan Technical University. Oct 28, 2021.
- Garcia Martin, H. "Guiding metabolic engineering via kinetic deep learning and multi-omics".
   Quantitative Modelling of Cell Metabolism Conference, October 20th, 2021.
- Garcia Martin, H. "Machine learning for industrial biotechnology". Delft Advanced Course Integrated Multi-Omics approaches for Improvement of Industrial Microbes, November 5th, 2021.





- Hillson, Nathan J. "Opportunities for CABBI collaboration with JBEI (and other DOE assets) in laboratory operations and data management infrastructure", CABBI seminar, via zoom, October 19, 2021
- Hillson, Nathan J. "Automation in Biotechnology: Challenges and Opportunities", Panelist at SENAI CETIQT (Brazil), via zoom, October 29, 2021
- Hillson, Nathan J. "ABF Overview and Capabilities", ABF Industry Day (via zoom), November 19, 2021
- Hector A Plahar, Stephen D. Lane, William C Morrell, Nathan J. Hillson and Christopher J. Petzold. "A Biological Parts Search Portal and Updates to the ICE Parts Registry Platform", JBEI Annual DOE Review, Dec 6-8, 2021
- Tijana Radivojevic, "Creating a Machine Learning Chassis to Maximize the Efficiency of the DBTL Cycle in Synthetic Biology", Synthetic Biology-Based Therapeutics Summit, December 9, 2021 (virtual)
- Hillson, Nathan J. "Overview and capabilities of the ABF and opportunities for collaboration with CSU", Colorado State Seminar Series (via zoom), February 3, 2022
- Hillson, Nathan J. "ABF prospective collaboration with other BETO consortia and projects", BETO Conversion Call (via MS Teams), March 7, 2022





- Bilbao, A. "Investigating deep learning approaches to advance data processing in Liquid Chromatography, Ion Mobility and Data-Independent Acquisition Mass Spectrometry Omics".
   70th ASMS Conference on Mass Spectrometry and Allied Topics, Minneapolis, Minnesota, June 9th, 2022.
- Dai Z., K.R. Pomraning, S. Deng, J. Kim, K.B. Campbell, A.L. Robles, and B.A. Hofstad, et al. 05/02/2022. "Assessment and Optimization of 3-hydroxypropionic acid production in industrial filamentous fungus-Aspergillus species." Presented by Z. Dai at 44th Symposium on Biotechnology for Fuels and Chemicals (SBFC 2022), New Orleans, Louisiana
- Adam Guss. "High efficiency DNA integration in diverse non-model microbes for rapid tool and pathway prototyping" SIMB SBFC. New Orleans, LA, May 3, 2022
- Magnuson, J.K.; Beckham, G.T.; Gladden, J.M.; Dale, T.; Guss, A.M.; Laible, P.; Hillson, N.J. 05/03/2022. "The Agile BioFoundry" Presented by Jon Magnuson at the 44th Symposium on Biotechnology for Fuels and Chemicals (SBFC 2022), New Orleans, Louisiana.
- Poirier, B.C. et al. "Mechanical cell disruption enhances the extraction of metabolites from bacterial and fungal species commonly used in metabolic engineering". 70th ASMS Conference on Mass Spectrometry and Allied Topics, Minneapolis, Minnesota, June 5-9, 2022.





- Poirier B.C. et al. "Behavior of lactam molecules during aqueous sample preparation and mass spectrometry analysis". 70th ASMS Conference on Mass Spectrometry and Allied Topics, Minneapolis, Minnesota, June 5-9, 2022.
- Hillson, Nathan J. "Overview and Capabilities of the DOE Agile BioFoundry", ABF Webinar (via zoom) April 29, 2022
- Hillson, Nathan J. "Overview and Capabilities of the DOE Agile BioFoundry", Lessafre visit to ESE (via zoom) May 6, 2022
- Hillson, Nathan J. "Overview and Capabilities of the DOE Agile BioFoundry" (Keynote Presentation) Bioindustry 4.0 – Synthetic Biology & Biofoundry, CHEY Institute – Scientific Innovation Series (via zoom) 12 May 17 (PDT) 18 (KST), 2022
- Hillson, Nathan J. "Overview and Capabilities of the DOE Agile BioFoundry", Wageningen University visit to ESE (via zoom) May 31, 2022
- Hillson, Nathan J. "ABF overview and DBTL Infrastructure", BETO Conversion Call (via MS Teams), June 13, 2022
- Nathan Hillson. "ABF Decarbonization Efforts", Invited Talk, LBL Carbon Negative Initiative –
   Biological Applications Lightning Talk Session (via zoom) 6/27/2022





- Radivojevic, Tijana. "Guiding synthetic biology via machine learning", Boston University, April 14, 2022, Guest lecture
- Deng S., J. Kim, K.R. Pomraning, Z. Dai, Y. Gao, N. Munoz Munoz, and Y. Kim, et al. 08/07/2022. "Identification of a specific exporter that enables high production of aconitic acid in A. pseudoterreus ATCC32359." Presented by S. Deng at SIMB annual meeting, San Francisco, California.
- Pomraning K.R., Z. Dai, S. Deng, N. Munoz Munoz, Y. Kim, B.A. Hofstad, and Y. Gao, et al. 08/10/2022. "Bioconversion of lignocellulosic feedstocks to 3-hydroxypropionic acid using acidophilic fungi." Presented by K.R. Pomraning at Society for Industrial Microbiology and Biotechnology, San Francisco, California.
- Jha RK. High throughput test tools for industrially relevant microbial chassis, SIMB 2022, San Francisco, August 2022 (invited talk).
- Adam Guss. "Using synthetic biology to solve challenges in plastic waste and renewable chemical production". Biological Sciences Departmental Seminar, Missouri S&T, Rolla, MO. September 27, 2022.
- Nathan Hillson. "Agile BioFoundry Connections with NSF, MSRDC, and Beyond", Invited Talk, LBL Biosciences Expert Advisory Committee: connections beyond Biosciences July 8, 2022





- Nathan Hillson. "Biosecurity concern anecdote", Screening Tools for Genome Engineering and Genome Editing (Inscripta-funded; Sarah Carter organized), via MS Teams, 9/9/2022
- Nathan Hillson. "Webinar Agenda and ABF Overview", ABF Webinar: Cutting-Edge
   Technologies for Accelerating Bioproduct Development at the Agile BioFoundry, 9/22/20222
- Garcia Martin, H. "Machine Learning and Automation for Predictive Synthetic Biology". Basque Center for Applied Mathematics, Bilbao, Spain, July 15th, 2022.



# License partners and patent applications

#### **Technologies licensed**

- Bioproduction of limonene from syngas
- Method to produce branched chain polyhydroxyalkanoates and branched chain 3-hydroxyacids

#### **Provisional Patents**

- ROI-18-92 U.S. provisional patent applications 63/163,518 63/321,207 63/479,918, not published
- ROI-21-104 U.S. provisional patent application 63/321,332
- ROI-21-63 U.S. provisional patent applications 63/163,518 63/321,207 63/479,918, not published

#### **Patent Applications**

- Post-transcriptional genome regulation in bacteria with next generation CRISPR-Cas tools
- · Terephthalate biosensor and applications thereof
- · Mutant transporters for bacterial uptake of terephthalic acid
- Alleviating the bottleneck in enzyme evolution and pathway optimization using novel biosensors (Disclosure Title) Modified Biosensors and Biocatalysts and Methods of Use (Application Title)
- Mutant transporters for bacterial uptake of terephthalic acid
- ART: A machine learning Automated Recommendation Tool for guiding synthetic biology





# Patent applications

#### **Patent Applications (cont.)**

- A Generative Model for Protein Sequences for the Purpose of Protein Design or Phenotypic Inference
- Predicting Metabolic Pathway Dynamics from Time Series Multiomics Data Using Machine Learning Techniques
- Use of Statistical Learn Approaches to Predict Next Generation Sequencing Subsequence Depth of Coverage
- Mutant transporters for bacterial update of terepthalic acid
- Method and strain for sugar conversion
- Engineered Microorganisms for the Production of Intermediates and Final Products (1st)
- Engineered Microorganisms for the Production of Intermediates and Final Products (2<sup>nd</sup>)
- Production of organic acids from Aspergillus pseduoterreus cadA deletion strain (1st)
- Production of organic acids from Aspergillus pseduoterreus cadA deletion strain (2<sup>nd</sup>)
- Genetically engineering an industrial filamentous fungus Aspergillus niger for 3hydroxypropionic acid production
- A specific exporter responsible for aconitic acid high production in Aspergillus pseduoterreus





#### Records of invention

#### **Records of Invention**

- Bioproduction of limonene from syngas
- Mutant transporters for bacterial update of terepthalic acid
- Method to produce branched chain polyhydroxyalkanoates and branched chain 3hydroxyacids
- A genetic circuit to reduce cell-to-cell production heterogeneity
- High yield conversion of D-xylose to D-arabitol in R. toruloides
- Manipulation of tRNA thiolation gene ncs2 for enhanced production of fatty-acyl-CoA derived chemicals in R. toruloides
- Efficient production of cis, cis-muconic acid from mixed substrates of glucose, D-xylose and L-arabinose
- Whole cell biosensors for industrially relevant polymers precursors
- Engineered Microorganisms for the Production of Intermediates and Final Products
- Method and strain for sugar conversion





# Software disclosures

#### **Software Disclosures**

- Automated Recommendation Tool (ART) v2.0
- Kinetic Learning v0.1
- Automated Recommendation Tool (ART): v1.0
- PIACE: Parallel Integration and Chromosomal Expansion of Metabolic Pathways
- OMG, Omics Mock Generator Library: v0.1.1
- Fermentation Data Processing
- Fermentation Data Manipulation and Analysis Once imported
- DIVA/Device Editor 3.1
- DIVA/Device Editor (DIVA) v6.0.0



